§ 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Specification:

Please substitute the following paragraphs for the pending paragraphs.

Substitute the paragraph bridging pages 3-4 with the following paragraph:

Another aspect of the invention provides a method for synthesizing nucleic acids, specifically DNA, using one or more enzymes, proteins or peptides (or fragments, mutants, derivatives or variants thereof) possessing RNase activity. Preferably, the ribonucleases used substantially lack DNase activity, and more preferably lack detectable levels of DNase activity. The method provided for synthesizing DNA (or other polynucleotides) comprises the step of mixing one or more desired templates with one or more enzymes, proteins or peptides (or fragments, mutants, derivatives or variants thereof) possessing RNase activity along with other reagents required for polynucleotide synthesis. Reagents required for polynucleotide synthesis include one or more nucleotides (e.g. dNTPs) or derivatives thereof, one or more polynucleotide primers, one or more DNA polymerases, and the like. The invention thus relates to a method of synthesizing a nucleic acid molecule comprising: (a) mixing a nucleic acid template with one or more DNA polymerases and with one or more RNases of the invention; and (b) incubating said mixture under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of said template. Thus, ribonuclease treatment may be conducted simultaneously with the nucleotide synthesis reaction and thus one or more ribonucleases may be added in conjunction with other components necessary for a nucleotide synthesis (e.g. nucleotides, primers, one or more DNA polymerases and the like). In a related aspect, one or more ribonucleases may

a1

be added to a sample prior to the nucleic acid synthesis step. Thus, a sample may be treated in accordance with the invention with one or more ribonucleases and following such treatment, nucleic acid synthesis in the presence of one or more polymerases may be conducted. In this aspect, the ribonuclease activity may or may not be inactivated after treatment but before synthesis by well known techniques. Thus, ribonuclease treatment may be accomplished prior to and/or during the nucleic acid synthesis reaction.

a' amt

Substitute the paragraph bridging pages 4-5 with the following paragraph:

Another aspect of the invention relates to amplification of nucleic acid molecules, for example a polymerase chain reaction or in an application of PCR, using one or more ribonucleases in accordance with the invention. The invention thus relates to a method for amplifying a double stranded DNA molecule, comprising: (a) providing a first and second primer, wherein said first primer is complementary to a sequence at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence at or near the 3'-termini of the second strand of said DNA molecule; (b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of one or more DNA polymerases and one or more RNases of the invention, under conditions such that a third DNA molecule complementary to said first strand and a fourth DNA molecule complementary to said second strand are synthesized; (c) denaturing said first and third strand, and said second and fourth strands; and (d) repeating steps (a) to (c) one or more times. For amplification of nucleic acid molecules, ribonuclease treatment may also be performed prior to and/or during nucleic acid synthesis or amplification. Thus, according to the invention, ribonucleases may be used at any step and may be removed or inactivated at any step. Removal or inactivation of ribonucleases can be accomplished using techniques well known to those of ordinary skill in the art (e.g. chemical extraction (phenol and/or chloroform), precipitation, protein denaturation, heat, etc.).

a²

Substitute the first paragraph on page 13 with the following paragraph:

Prior to the inventors' work, polynucleotide synthesis *in vitro* was performed without RNase. In a variety of nucleic acid synthesis procedures, the subject compositions provide superior synthesis results, as compared with synthesis results obtained without RNase. The composition is especially useful in DNA synthesis when the sample is crude, i.e. prepared rapidly such that it contains contaminating RNA. In such situations, the result achieved, i.e., the amount of synthesis product produced, is significantly greater than the amount of synthesis product obtained without RNase. Other advantages of the subject compositions and methods include increased product length, as well as the synthesis of polynucleotides that could not be synthesized previously, i.e., in the absence of RNase.

a3

Substitute the second paragraph on page 13 with the following paragraph:

The subject invention thus provides novel compositions ~~for use in synthesizing~~ nucleic acids, particularly DNA. The subject compositions comprise one or more ribonucleases and may optimally further comprise one or more DNA polymerases. Such compositions may also comprise one or more components selected from the group consisting of one or more nucleotides, one or more primers, one or more buffers suitable for nucleic acid synthesis and/or one or more templates.

a4

Substitute the paragraph bridging pages 13-14 with the following paragraph:

Enzymes, proteins or peptides (or fragments, mutants, variants or derivatives thereof) possessing RNase activity for use in the present compositions and methods may be isolated from natural sources, produced through recombinant DNA techniques, or chemically synthesized. Such enzymes that possess RNase activity and their properties are detailed in The Enzymes, Vol. IV (P.D. Boyer, ed.) Academic Press, San Diego. Examples of enzymes that possess RNase activity useful in the compositions and methods of the present invention include RNase A, RNase H, RNase T1, RNase T2, RNase S, RNase B, RNase C or variants, derivatives, fragments or mutants thereof and the like.

a5

Substitute the first full paragraph on page 14 with the following paragraph:

a6

RNase A, a preferred enzyme for use in the present invention, is an endoribonuclease from bovine pancreas that hydrolyzes RNA after C (cytosine) and U (Uracil) residues (Richard and Wyckoff (1971) The Enzymes, Vol. IV (P.D. Boyer, ed.) pp. 647-806. Academic Press, San Diego). Cleavage occurs between the 3'-phosphate group of a pyrimidine ribonucleotide and the 5'-hydroxyl of the adjacent nucleotide. The reaction generates a 2':3' cyclic phosphate which then is hydrolyzed to the corresponding 3'-nucleoside phosphate.

Substitute the second full paragraph on page 14 with the following paragraph:

a7

Ribonuclease T1 from *Aspergillus oryzae* is an endoribonuclease that hydrolyzes RNA after G residues (Uchida and Egami (1971) The Enzymes, Vol. IV (P.D. Boyer, ed.) pp. 205-250. Academic Press, San Diego). Cleavage occurs between the 3'-phosphate group of a guanine ribonucleotide and the 5'-hydroxyl of the adjacent nucleotide. The reaction generates a 2':3' cyclic phosphate which then is hydrolyzed to the corresponding 3'-nucleoside phosphate.

Substitute the third full paragraph on page 15 with the following paragraph:

a8

RNase H from *E. coli* is an endoribonuclease that specifically hydrolyzes the phosphodiester bonds of RNA in RNA:DNA duplexes to generate products with 3'hydroxyl and 5'phosphate ends (Berkower *et al.* (1973) *J. Biol. Chem.* 248:5914-5924).

Substitute the paragraph bridging pages 17-18 with the following paragraph:

a9

A variety of polypeptides having polymerase activity are useful in accordance with the present invention. Included among these polypeptides are enzymes such as nucleic acid polymerases (including DNA polymerases). Such polymerases include, but are not limited

to, *Thermus thermophilus (Tth)* DNA polymerase, *Thermus aquaticus (Taq)* DNA polymerase, *Thermotoga neopolitana (Tne)* DNA polymerase, *Thermotoga maritima (Tma)* DNA polymerase, *Thermococcus litoralis (Tli* or VENT®) DNA polymerase, *Pyrococcus furiosus (Pfu)* DNA polymerase, *Pyrococcus* species GB-D (DEEP VENT™) DNA polymerase, *Pyrococcus woosii (Pwo)* DNA polymerase, *Bacillus sterothermophilus (Bst)* DNA polymerase, *Bacillus caldophilus (Bca)* DNA polymerase, *Sulfolobus acidocaldarius (Sac)* DNA polymerase, *Thermoplasma acidophilum (Tac)* DNA polymerase, *Thermus flavus (Tfl / Tub)* DNA polymerase, *Thermus ruber (Tru)* DNA polymerase, *Thermus brockianus* (DYNAZYME™) DNA polymerase, *Methanobacterium thermoautotrophicum (Mth)* DNA polymerase, mycobacterium DNA polymerase *(Mtb, Mlep)*, and mutants, variants and derivatives thereof.

Substitute the paragraph bridging pages 18-19 with the following paragraph:

Polymerases used in accordance with the invention may be any enzyme that can synthesize a nucleic acid molecule from a nucleic acid template, typically in the 5' to 3' direction. The nucleic acid polymerases used in the present invention may be mesophilic or thermophilic, and are preferably thermophilic. Preferred mesophilic DNA polymerases include T7 DNA polymerase, T5 DNA polymerase, Klenow fragment DNA polymerase, DNA polymerase III and the like. Preferred thermostable DNA polymerases that may be used in the methods of the invention include *Taq*, *Tne*, *Tma*, *Pfu*, *Tfl*, *Tth*, Stoffel fragment, *Tli* (VENT®) and *Pyrococcus* species GB-D (DEEP VENT™) DNA polymerases, and mutants, variants and derivatives thereof (U.S. Patent No. 5,436,149; U.S. Patent No. 4,889,818; U.S. Patent No. 4,965,188; U.S. Patent No. 5,079,352; U.S. Patent No. 5,614,365; U.S. Patent No. 5,374,553; U.S. Patent No. 5,270,179; U.S. Patent No. 5,047,342; U.S. Patent No. 5,512,462; WO 92/06188; WO 92/06200; WO 96/10640; Barnes, W.M., *Gene 112*:29-35 (1992); Lawyer, F.C., *et al., PCR Meth. Appl. 2*:275-287 (1993); Flaman, J.-M, *et al., Nucl. Acids Res. 22*(15):3259-3260 (1994)). For amplification of long nucleic acid molecules (e.g., nucleic acid molecules longer than about 3-5 Kb in length), at least two DNA polymerases (one substantially lacking 3' exonuclease activity and the other having 3' exonuclease activity) are typically used. See U.S. Patent No.

5,436,149; U.S. Patent No. 5,512,462; Barnes, W.M., *Gene 112*:29-35 (1992), the disclosures of which are incorporated herein in their entireties. Examples of DNA polymerases substantially lacking in 3' exonuclease activity include, but are not limited to, *Taq, Tne*(exo$^-$), *Tma*(exo$^-$), *Pfu*(exo$^-$), *Pwo*(exo$^-$) and *Tth* DNA polymerases, and mutants, variants and derivatives thereof.

a10 cont

Substitute the paragraph bridging pages 21-22 with the following paragraph:

When sequencing a DNA molecule, ddNTPs lack a hydroxyl residue at the 3' position of the deoxyribose base and thus, although they can be incorporated by DNA polymerases into the growing DNA chain, the absence of the 3'-hydroxy residue prevents formation of the next phosphodiester bond resulting in termination of extension of the DNA molecule. Thus, when a small amount of one ddNTP is included in a sequencing reaction mixture, there is competition between extension of the chain and base-specific termination resulting in a population of synthesized DNA molecules which are shorter in length than the DNA template to be sequenced. By using four different ddNTPs in four separate enzymatic reactions, populations of the synthesized DNA molecules can be separated by size so that at least a part of the nucleotide sequence of the original DNA molecule can be determined. DNA sequencing by dideoxy-nucleotides is well known and is described by Sambrook *et al.*, *Molecular Cloning, a Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). As will be readily recognized, the polymerases of the present invention may be used in such sequencing reactions.

a11

Substitute the first full paragraph on page 23 with the following paragraph:

The invention also relates to amplification or synthesis of cDNA. As is known, cDNA is prepared from mRNA templates. See U.S. Patent Nos. 5,405,776 and 5,244,797. The double stranded cDNA is typically cloned into a host cell and such host cells may be used in the present invention.

a12

Substitute the paragraph bridging pages 24-25 with the following paragraph: